Amphetamine Promotes Recovery From Sensory-Motor Integration Deficit After Thrombotic Infarction of the Primary Somatosensory Rat Cortex

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The present studies were undertaken to examine 1) whether d-amphetamine sulfate administered to rats well after thrombotic infarction of the vibrissal cortical barrel-field within the primary somatosensory cortex affected the rate and completeness of behavioral recovery and 2) whether a dose–response relation exists between d-amphetamine sulfate dose and recovery of function. In a learning task requiring sensory–motor integration, 41 rats were trained to perform a motor response in a T-maze consequent to the detection of a vibrissal deflection cue. Once training was complete, unilateral (n=29) or sham (n=12) infarction was produced by a noninvasive photochemical technique. After infarction, T-maze performance was assessed repeatedly in rats receiving 2 (n=10) or 4 (n=10) mg/kg d-amphetamine sulfate or saline (n=9) 24 hours prior to testing on days 4, 6, 9, and 11. The sham-operated control rats received d-amphetamine sulfate (n=7) or no injections (n=5). All three infarcted groups displayed a reliable and sustained behavioral deficit in performance that was not present in the sham-operated control animals. Although the performance of each infarcted group improved over the testing sessions after the first injection, the amphetamine-treated groups improved at a faster rate than the saline-injected group. The results further demonstrated a dose–response effect, with the 4 mg/kg amphetamine group recovering to within preinfarction levels 6–8 days earlier than the 2 mg/kg amphetamine and saline-injected groups. Moreover, both amphetamine-treated groups recovered more completely than the saline-injected group. Quantification of the chronic infarct area revealed no differences among the amphetamine-treated and saline-injected groups. These data provide further evidence of the facilitatory effect of d-amphetamine sulfate on recovery from brain injury and extend this effect to the enhancement of recovery subsequent to thrombotic infarction of the primary somatosensory cortex. (Stroke 1991;22:648–654)

Since the turn of this century, the remarkable capacity for the brain to recover from cerebral injury, such as stroke, has spurred interest in the use of animal models to study mechanisms of functional recovery and apply them in the clinical setting. In recent years, data from animal studies and preliminary clinical findings indicate that some pharmacologic manipulations that alter neurotransmitter (e.g., acetylcholine, γ-amino butyric acid, serotonin, dopamine, and norepinephrine [NE]) function may markedly promote recovery of function following brain injury. One promising agent, the catecholamine agonist amphetamine, has been consistently shown during the past 20 years to reinstate locomotor, righting, and other postural reflexes and enhance recovery from learning and memory deficits induced by electrolytic brain lesions. In particular, one recent study found that a single dose of amphetamine given to cats 10 days after unilateral motor cortex ablation accelerated the rate of recovery of beamwalking ability compared with saline. When amphetamine was injected at 4-day intervals from the 10th to
accompanying increase in the rate of recovery was produced
with water available ad libitum. The rats were ran-
tomly assigned, with 29 receiving unilateral infarc-
tion, 12 sham-operated rats. All injections received 2
mg/kg i.p. (d-AMP) (Sigma Chemical Co., St. Louis, Mo.) (n = 10), 4 mg/kg i.p.
d-AMP (n = 10), or intraperitoneal saline (n = 9).
Sham-operated rats received either 2 or 4 mg/kg i.p.
d-AMP (n = 7) or no injections (n = 5). The control
group comprises all 12 sham-operated rats. All injec-
tions were given approximately 24 hours prior to
the third day to establish whether a behavioral deficit
was present. Infarcted animals were then divided
randomly into three treatment groups and received 2
mg/kg i.p. d-amphetamine sulfate (d-AMP) (Sigma
Chemical Co., St. Louis, Mo.) (n = 10), 4 mg/kg i.p.
d-AMP (n = 10), or intraperitoneal saline (n = 9).
Sham-operated rats received either 2 or 4 mg/kg i.p.
d-AMP (n = 7) or no injections (n = 5). The control
group comprises all 12 sham-operated rats. All injec-
tions were given approximately 24 hours prior to
testing on each of postsurgical days 4, 6, 9, and 11.
Experimenter's were blinded to the surgical and
pharmacologic treatment each animal received.

Photochemical cerebral infarction was induced
only after the behavioral criterion was reached. A
detailed account of the apparatus and procedure for
inducing photochemical infarction has been de-
scribed. Briefly, in the infarcted groups, photo-
chemical infarction was induced in halothane-anes-
thetized rats by infusing 20 mg/kg i.v. rose bengal dye
through a tail vein catheter for 2 minutes and then
irradiating the cranium for 7 minutes at 7.2 mm
anterior to the interaural line and 5.0 mm lateral to
the midline directly over the left cortical barrel-field
of the primary somatosensory cortex. Sham-oper-
ated rats underwent the same procedure as the
infarcted animals, except isotonic saline instead of
rose bengal dye was infused and consequently no
photochemical reaction took place.

At the end of the study, 40–45 days after surgery,
the rats were deeply anesthetized with halothane and
perfusion-fixed with formaldehyde, glacial acetic acid, and methanol. The brains were left in situ overnight before removal from the cranial vault. The brains were then stored in the perfusion solution until they were blocked, dehydrated, and infiltrated with and embedded in paraffin. Coronal sections 10 μm thick were cut and stained using hematoxylin and eosin. The lesion's epicenter and the area of maximal cortical necrosis were first determined by light microscopy of multiple stained sections. The tracing of each histologic section was subsequently redrawn onto a digitizing tablet (Summagraphics Corp., Seymour, Conn.) interfaced with a MicroVax minicomputer, which computed the areas.

The data were assessed to determine whether d-AMP administration affected T-maze task performance relative to baseline performance. The mean percentage of correct responses for the three baseline sessions was subtracted from the percentage of correct responses for each postsurgical session to obtain an index of performance relative to baseline. Behavioral deficit was established as the difference between the mean baseline percentage and the postsurgical day 3 percentage of correct responses. Behavioral recovery was assessed using the BMDP computer statistical package with repeated-measures analysis of variance. Analyses were performed to assess differences between groups (2 mg/kg d-AMP, 4 mg/kg d-AMP, saline, and sham-operated controls) in the rate of recovery over postsurgical days 3 to 35. An additional measure of behavioral recovery was obtained by comparing the postsurgical day when performance recovered to within 10% of baseline to assess the temporal influence of d-AMP treatment.

Results

A consistent pattern of cortical necrosis was demonstrated in both d-AMP- and saline-treated rats. Figure 1 displays sections through the infarct epicenter (7.2 mm anterior to the interaural line) from representative d-AMP- and saline-treated rats. Infarcts were well-demarcated and appeared cystic. In addition to a glial scar, the infarct contained macrophages, astrocytes, and blood vessels. The chronic infarcts commonly extended 9.2 mm anterior and 4.7 mm posterior of the interaural line; the medial border was 4.0 mm lateral to the midline, and the lateral border was 5.0 mm dorsal to the interaural line. Quantitative analysis of infarct areas demonstrated no significant differences between saline-treated (2.5±0.2 mm²) and d-AMP-treated (2 mg/kg 2.0±0.2 mm², 4 mg/kg 2.2±0.2 mm²) rats.

Analysis of baseline performance showed no significant differences between the infarcted and control groups. The mean±SEM percentage of correct responses (baseline performance) for all groups was 89.0±0.6%.

Figure 2 depicts the change in mean percentage of correct responses per session from postsurgical day 3 to 35 for all groups. The analysis of behavioral deficit comparing the infarcted and sham-operated groups' performance during baseline with postsurgical day 3 performance revealed a significant interaction between groups over testing days (F(3,37)=10.8, p<0.001). No difference among the sham-operated subgroups was found (data not shown), although a small but significant behavioral deficit was observed (F(1,9)=13.6, p<0.01). The infarcted groups combined also displayed a significant behavioral deficit (F(1,26)=277.3, p<0.001), obtaining only 61.6% of the trials correct, a behavioral performance at or just above the level of chance. An analysis of only the postsurgical day 3 performance of the infarcted groups combined revealed a greater behavioral deficit (27.0±1.6%) than the control group (9.9±2.9%) (F(1,39)=33.6, p<0.001; Figure 2).

Analysis of the effect of d-AMP on the control group's performance from day 3 to 35 revealed no significant subgroup differences, and therefore data for the 2 mg/kg d-AMP, 4 mg/kg d-AMP, and no injection sham-operated rats were combined. Rates of recovery of performance over postsurgical days 3 to 35 differed in the control and infarcted groups (F(3,37)=21.2, p<0.001; Figure 2). The behavioral deficit in the control group was short-lived, and by day 5 (the second testing session after sham surgery), per-
performance did not differ significantly from baseline. In contrast, the behavioral deficit of the infarcted groups was more prolonged, lasting 1–2 weeks, before performance gradually recovered toward baseline (Figure 2). Repeated-measures analysis of variance of the three infarcted groups' performance from day 3 to 35 yielded a significant interaction between treatment and postinfarction day ($F_{(2,30)}=3.5, p<0.05$). When the two d-AMP-treated groups were compared from day 3 to 35, no differences emerged, although a significant increasing linear trend over postinfarction days was found ($F_{(1,18)}=214.1, p<0.001$), indicating that the two d-AMP-treated groups recovered at the same rate. However, when the saline-treated group was compared with either d-AMP-treated group, significant differences in the recovery of performance over postinfarction days was observed ($F_{(4,7)}=5.4, p<0.05$ for 2 mg/kg d-AMP; $F_{(4,7)}=4.5, p<0.05$ for 4 mg/kg d-AMP). Therefore, d-AMP at either dose resulted in greater acceleration of the rate at which performance improved after unilateral cerebral infarction compared with saline.

Figure 3 displays the mean±SEM postsurgical day when performance recovered to within 10% of baseline for all groups. The analysis of these data revealed a significant difference among groups ($F_{(3,37)}=33.3, p<0.001$). The control group recovered by about the second postsurgical testing session (5.1±0.6 days). When the infarcted groups were compared, a significant treatment effect ($F_{(2,17)}=3.7, p<0.05$) was observed. As seen in Figure 3, the 4 mg/kg d-AMP group recovered sooner than the saline-treated group ($F_{(2,17)}=6.2, p<0.05$) or the 2 mg/kg d-AMP group ($F_{(2,18)}=6.2, p<0.05$). No significant difference was found between the saline-treated and 2 mg/kg d-AMP groups. Therefore, the high-dose d-AMP group recovered to within 10% of baseline significantly earlier (19.4±1.4 days) than either the low-dose d-AMP (25.6±2.0 days) or the saline-treated (27.5±3.0 days) group.

To determine whether d-AMP affected the overall level of recovery by the end of the testing period, another analysis assessed differences between groups from day 33 to 35. Both d-AMP-treated groups displayed a more complete behavioral recovery than the saline-treated group ($F_{(2,17)}=4.57, p<0.05$ for 2 mg/kg d-AMP and $F_{(2,18)}=4.41, p<0.05$ for 4 mg/kg d-AMP). Mean±SEM changes on days 33–35 from baseline of 0.4±1.9%, 0.2±1.4%, and 8.1±3.7% were observed for the 4 mg/kg d-AMP, 2 mg/kg d-AMP, and saline-treated groups, respectively (Figure 2).

**Discussion**

Using combined pharmacological and behavioral strategies in the present study, we show that d-AMP, when administered in multiple injections well after the cerebrovascular insult, facilitates behavioral recovery from a sensory–motor integration deficit sub-
sequent to thrombotic infarction of the primary somatosensory cortex in rats. Unilateral infarction of the cortical barrel-field contralateral to vibrissal stimulation produced a large decrease in the percentage of correct behavioral responses, with the accuracy of the infarcted animals dropping to the level of chance at the first postinfarction session. The behavioral deficit exhibited by these rats was not due to any motor deficit since they still performed the task, albeit inaccurately. Moreover, the behavioral deficit probably did not result from a loss of the animals' ability to sense vibrissal deflection since, in a previous study, rats with unilateral or bilateral lesions of the primary somatosensory cortex were still able to discriminate vibrissal deflections, as indicated by the reflexive inhibition of an ongoing motor behavior. Instead, the inability to produce the correct motor response was more akin to a vibrissal agnosia; the rats simply were unable to integrate the vibrissal deflection with the correct motor response. The saline-treated infarcted animals in this study displayed a gradual improvement in performance from postinfarction days 3 through 35, recovering to within 10% of baseline between 25 and 35 days following cortical injury. It should be noted that in previous studies, as well as in the current study, although there was a significant improvement in performance, the infarcted animals did not recover completely. Thus, some performance deficit remained long after the cerebral ischemic event was induced.

The major focus of this study was whether d-AMP influenced the recovery of function after cerebral infarction of the primary somatosensory cortex. At both 2 and 4 mg/kg, d-AMP augmented the rate of behavioral recovery relative to saline. Moreover, the high-dose d-AMP group recovered to within 10% of baseline earlier, by the 19th day following infarction, than the low-dose d-AMP and saline-treated groups, which recovered to within 10% of baseline by about days 26 and 28, respectively. Recovery in the d-AMP-treated animals not only approached, but matched, baseline, indicating more complete recovery than the saline-treated rats, in which performance remained about 8% below baseline. Therefore, behavioral recovery occurred more quickly and more completely in the infarcted rats receiving d-AMP than in those receiving saline.

The ability of the high dose of d-AMP to accelerate behavioral recovery is consistent with previous findings of d-AMP-accelerated behavioral recovery from motor cortex ablations in both rats and cats performing motor tasks requiring more basic and complex initiation and modulation of movement. More recent studies have found that single or multiple injections of 2 mg/kg d-AMP given after unilateral motor cortex ablation enhanced the rate of recovery of beam-walking compared with saline, with multiple injections yielding a more rapid recovery than a single dose. A recent dose–response study employing this same behavioral task reported that d-AMP affects recovery over a narrow range of doses. The d-AMP dose with optimal effect on beam-walking recovery was 3.5 mg/kg; lower doses had less or no effect on recovery, and higher doses elicited behavioral stereotypes that were suggested to interfere with locomotion, and consequently high-dose d-AMP was less effective in modifying recovery. These dose–response findings corroborate ours; we found a greater facilitation of behavioral recovery with 4 mg/kg d-AMP than with 2 mg/kg d-AMP.

The relatively high doses of d-AMP required to promote recovery can affect NE, dopamine, and serotonin neurotransmission, thus implicating these substances in postinjury recovery. Adrenergic agonists activate locus ceruleus noradrenergic neurons, resulting in sustained stimulation of cortical NE release and neuronal excitability. The beneficial effects of d-AMP on rat motor recovery are blocked by haloperidol, a D2 dopamine receptor–blocking agent, which is often given to counter agitation in stroke patients. Thus, dopamine may play a role in d-AMP-facilitated recovery. Consistent with this notion are data showing that the D2 agonist bromocriptine may enhance functional recovery following brain injury. However, it is possible that the beneficial effect of dopamine is the result of its being taken up by presynaptic NE terminals and metabolized to NE. The dopamine agonists apomorphine and methylenidate have weak or no effects on motor recovery. In contrast, the α1-adrenergic antagonists idazoxan and yohimbine, but not the α1-adrenergic antagonist prazosin nor the β-adrenergic antagonist propranolol, improved motor function following unilateral sensory-motor cortical damage. Indeed, a single dose of NE enhanced motor recovery but has only a transient facilitatory effect if the adrenergic nucleus, the locus ceruleus, has been previously lesioned. Taken together, these findings suggest that amphetamine-accelerated recovery may be mediated by noradrenergic rather than dopaminergic neurotransmission.

Since d-AMP-treated rats in beam-walking studies appear to recover by the first testing session, it is improbable that the behavioral recovery is due to structural changes proximal to the site of injury. Alternatively, the behavioral deficit observed in beam-walking may, as has been previously suggested, be the result of a generalized depression of subcortical structures related to the motor pathways that were required to perform the motor response. That is, the initial behavioral deficit may arise from the lesioned area and functional depression of neural structures remote from but connected to the site of injury. Studies in our laboratory using the photochemical model of cerebral infarction have revealed a temporal pattern of widespread pathophysiological change. Following unilateral cortical infarction, consistent with the notion of remote functional depression, an acute decline of hemodynamic and metabolic function occurs within
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